

THAT WHICH IS CLAIMED IS:

1. A method of amplifying an mRNA, comprising the steps of:
 - (a) binding a first primer to a target mRNA, the first primer comprising, in the
5 5' to 3' direction, a first known segment and an oligo T segment;
 - (b) transcribing a cDNA from said target mRNA by elongation of said first
primer with reverse transcriptase; and then
 - (c) linking a second known segment to the 3' terminus of said cDNA.
- 10 2. The method of claim 1, wherein:
said step of transcribing a cDNA from said target mRNA is carried out so that
at least one additional C residue is produced on the 3' terminus of said cDNA; and
said step of linking a second known segment to the 3' terminus of said cDNA
is carried out by:
 - 15 (i) binding a second bridge primer to said cDNA, said second primer
comprising, in the 5' to 3' direction, a second known segment and at least one
G residue, said second primer having an inactivated G residue on the 3'
terminus thereof; and then
 - (ii) further transcribing said cDNA from second bridge primer by
20 elongation of said at least one additional C residue with reverse transcriptase
so that a cDNA is produced having said first known segment on the 5'
terminus thereof and said second known segment on the 3' terminus thereof.
3. The method of claim 1, wherein:
 - 25 said step of transcribing a cDNA from said target mRNA is followed by the
step of adding at least one additional predetermined residue to the 3' terminus of said
cDNA with a terminal deoxynucleotidyl transferase; and
 - said step of linking a second known segment to the 3' terminus of said cDNA
is carried out by:
 - 30 (i) binding a second bridge primer to said cDNA, said second primer
comprising, in the 5' to 3' direction, a second known segment and at least one
corresponding residue, which corresponding residue binds to said at least one
additional predetermined residue by Watson-Crick pairing, said second primer

having an inactivated predetermined residue on the 3' terminus thereof; and then

5 (ii) further transcribing said cDNA from second bridge primer by elongation of said at least one additional predetermined residue with reverse transcriptase so that a cDNA is produced having said first known segment on the 5' terminus thereof and said second known segment on the 3' terminus thereof.

10 4. The method of claim 3, wherein said at least one additional unmatched residue is selected from the group consisting of A, T, C, G, and oligomers thereof, and said at least one corresponding residue is selected from the group consisting of A, T, C, G, and oligomers thereof.

15 5. The method of claim 3, wherein said at least one additional unmatched residue is selected from the group consisting of C and oligo C, and said at least one corresponding residue is selected from the group consisting of G and oligo G.

20 6. The method of claim 1, wherein said step of linking a second known segment to the 3' terminus of said cDNA is carried out by directly linking said second known segment to said 3' terminus with RNA ligase.

 7. The method of claim 6, wherein said second known segment comprises a DNA.

25 8. The method according to claim 1, further comprising the step of:
 (e) amplifying said cDNA with a pair of primers, one of which pair binds to said first known segment and the other of which pair binds to said second known segment.

30 9. The method according to claim 8, wherein said amplifying step comprises a polymerase chain reaction amplification step.

10. The method according to claim 8, wherein said amplifying step comprises an amplification process selected from the group consisting of strand displacement amplification, rolling circle amplification, and fluorescent oligonucleotide dendrimeric signal amplification.

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11. A method of uniformly amplifying a plurality of different target mRNAs in a sample, said method comprising the steps of:

- (a) binding a first primer to a each of said target mRNA, the first primer comprising, in the 5' to 3' direction, a first known segment and an oligo T segment;
- 10 (b) transcribing a cDNA from said each of said target mRNA by elongation of said first primer with reverse transcriptase; then
- (c) linking a second known segment to the 3' terminus of each of said cDNAs; and then
- (d) uniformly amplifying each of said cDNAs with a pair of primers, one of
- 15 which pair binds to said first known segment and the other of which pair binds to said second known segment.

12. The method of claim 11, wherein:

20 said step of transcribing a cDNA from each of said target mRNA is carried out so that at least one additional unmatched C residue is produced on the 3' terminus of said cDNAs; and

said step of linking a second known segment to the 3' terminus of each said cDNAs is carried out by:

- (i) binding a second bridge primer to each of said cDNAs, said second
- 25 primer comprising, in the 5' to 3' direction, a second known segment and at least one G residue, said second primer having an inactivated G residue on the 3' terminus thereof; and then
- (ii) further transcribing said cDNAs from second bridge primer by elongation of said at least one additional C residue with reverse transcriptase
- 30 so that a plurality of cDNAs is produced having said first known segment on the 5' terminus thereof and said second known segment on the 3' terminus thereof.

13. The method of claim 11, wherein:

said step of transcribing a cDNA from each of said target mRNAs is followed
by the step of adding at least one additional predetermined residue to the 3' terminus
5 of each of said cDNAs with a terminal deoxynucleotidyl transferase; and

said step of linking a second known segment to the 3' terminus of said cDNAs
is carried out by:

(i) binding a second bridge primer to each of said cDNAs, said second
primer comprising, in the 5' to 3' direction, a second known segment and at
10 least one corresponding residue, which corresponding residue binds to said at
least one additional predetermined residue by Watson-Crick pairing, said
second primer having an inactivated residue on the 3' terminus thereof; and
then

(ii) further transcribing said cDNAs from second bridge primer by
15 elongation of said at least one additional predetermined residue with reverse
transcriptase so that a plurality of cDNAs is produced having said first known
segment on the 5' terminus thereof and said second known segment on the 3'
terminus thereof.

20 14. The method of claim 13, wherein said at least one additional
predetermined residue is selected from the group consisting of A, T, C, G, and
oligomers thereof, and said at least one corresponding residue is selected from the
group consisting of A, T, C, G, and oligomers thereof.

25 15. The method of claim 13, wherein said at least one additional
predetermined residue is selected from the group consisting of C and oligo C, and said
at least one corresponding residue is selected from the group consisting of G and oligo
G.

30 16. The method of claim 11, wherein said step of linking a second known
segment to the 3' terminus of said plurality of cDNAs is carried out by directly
linking said second known segment to said 3' terminus with RNA ligase.

17. The method of claim 16, wherein said second known segment comprises a DNA.

18. The method according to claim 11, wherein said amplifying step
5 comprises a polymerase chain reaction amplification step.

19. The method according to claim 11, wherein said amplifying step comprises an
amplification process selected from the group consisting of strand displacement
amplification, rolling circle amplification and fluorescent oligonucleotide dendrimeric
10 signal amplification.

20. The method according to claim 11, wherein said target mRNAs consists
essentially of mRNA extracted from not more than 100 cells.

15 21. The method according to claim 11, wherein said target mRNAs consists
essentially of not more than 10 nanograms of mRNA.

22. The method according to claim 11, further comprising the step of:

(e) determining the quantity of each of at least a portion of said cDNAs to
20 thereby provide an indication of the relative amounts of the corresponding mRNAs
present in said sample.

23. The method according to claim 11, wherein:

said target mRNAs consist essentially of mRNA extracted from not more than
25 100 cells; said method further comprising the step of:

(e) determining the quantity of each of at least a portion of said cDNAs to
thereby provide an indication of the relative amounts of the corresponding mRNAs
present in said sample.

30 24. The method according to claim 11, wherein said first known segment and
said second known segment comprise the same nucleic acid sequence in opposite
orientation, and wherein said amplifying step comprises a rolling circle amplification
reaction.

25. A primer pair useful for amplifying an mRNA, comprising:

(a) a first primer, said first primer comprising, in the 5' to 3' direction, a first known segment and an oligo T segment; and

5 (b) a second bridge primer, said second primer comprising, in the 5' to 3' direction, a second known segment and a binding segment, said binding segment selected from the group consisting of A, oligo A, T, oligo T, C, oligo C, G, and oligo G, said second primer having an inactivated residue on the 3' terminus thereof.

10 26. The primer pair according to claim 25, wherein said binding segment comprises an oligo C segment.

27. A kit comprising a primer pair according to claim 25.

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